Chemical Composition and Larvicidal Activities of Essential Oils of Medicinal Plants, *Artemisia Sieberi* and *Tanacetum Balsamita* Against Malaria Vector, *Anopheles Stephensi*

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Research

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Abstract

Background: The country is suffering from malaria disease. There are several chemical control of mosquito. Due to resistant of vectors to different pesticides, this research was conducted to measure the chemical components and larvicidal activities of two native plants, *Artemisia sieberi* and *Tanacetum balsamita* against *Anopheles stephensi*.

Materials and methods: Two species of medicinal plants were collected from different localities in in Iran. The plant samples were dried in shaded place and Essential Oils (EO) dried over anhydrous sodium sulfate. The EOs was maintained in the dark sealed vials until conduction larvicidal tests. The maximum storing of EOs was two days. The larvicidal tests were carried out based on the guideline of WHO.

Results: The values of LC50 and LC90 of EOs were 47.9 and 178.8 ppm for *Artemisia sieberi*. The figure of 26.2and 52.4 ppm was observed for *Tanacetum balsamita*.The chemical constituents of *Tanacetum balsamita* and *Artemisia sieberi* which showed the highest efficacy for larviciding. A total of 39 constituents were isolated from *Tanacetum balsamita* .The main constituents were Thujone (52.37%) and Carvone (26.84%). Totally 57 constituents were detected in *Artemisia sieberi* and the main components were : camphor (23.6%) , 2-Ethyl-3- methyl maleic anhydride (15. 193%) and, Bombykal (10.32%) , Ethylbutenol (10.74%) .

Conclusion: New formulations of plants should be prepared and then evaluated under semi-filed and filed conditions in a malrious areas.

Background

Mosquitoes are serious public health problem and important pests in tropical and sub-tropical countries. *Anopheles stephensi*, a highly competent vector of plasmodium is considered a prominent vector of urban malaria [1]. Malaria is the main vector borne diseases worldwide. According to the recent record of World Health Organization, 228 million cases have been reported in 2018 mainly in in African region [2]. Based on the report of Ministry of Health of Iran, less than 89 locally-transmitted cases in 2017 have been reported. The aim of country is to eliminate the disease by 2025 [3]. *An. stephensi* is the main malaria vector southern parts of country [4]. Vector control by conventional insecticides, personal protection and using repellents from mosquito bites are the best measures of reducing the transmission of these diseases to human. The chemical insecticides have various problems such as, development of resistance, environment pollution and toxic effects on human and non-target organisms [5]. Malaria control in the country is now based on use different insecticides. Distribution of impregnated bed net with permethrin. Using *Bacillus thurngiensis* and Temephos as larvicide. (CDC, Ministry of Health). According to the WHO (2016) [6] report, drug and insecticide resistance in the malaria parasites and Anopheles species is the main problem. In malarious areas of the world, current vector control measure is not able to control the diseases effectively. Therefore, there is a need to develop and replace these toxic insecticides with new and better ones which are more environmentally safe. In Iran there are some species of malaria

vectors including: *An. stephensi*, *An. dthali*, *An. culicifacies*, *An. fluviatilis*, *An. superpictus* s.l., *An. sacharovi*, *An. maculipennis* Complex (Fig. 1) .

Materials And Methods

Collection, identification and extraction of plants: The plants were collected in East Azerbaijan Province, Northern Iran (Fig.2) . Subsequently they were rapidly transported to the School of Public Health, Tehran University of Medical Sciences

Plant identification: The plant was identified by experts in Department of Plant Sciences, Tehran University (Figs 3,4).

Rearing of mosquito larvae

Rearing and maintaining mosquito larvae was carried out in the Culicidae insectarium of the School of Public Health Tehran University of Medical Sciences under standard conditions. Early 3^{rd}-4^{th} instar of larvae were used for larvicidal activities.

Biological tests (larvicidal)

For larvicidal activities of EO of plants, the standard WHO method was used. Late 3rd - 4th instar larvae were used. In each test, 5 logarithmic concentrations of larvicide were chosen. A total of 4 replicates for each concentration and 2 replicated as control were considered.

Statistical analysis

The test results of evaluation of EO of plants were calculated after 24hours exposure. The concentrations which cause 50 and 90% mortality were calculated as LC50 and LC90. Their confidence intervals and the regression line were measured using a regression probit analysis according to the Finney (1971) (7). The percentage mortality was calculated according to Abbot's formula (1925) (8).

Extraction of essential oil of plant

Faculty of Pharmacology, Tehran University of Medical Sciences were involved in all plant extractions . Essential oils (EOs) of native medicinal plants were hydro distilled in a Clevenger- for 4-6 h and then anhydrous sodium sulfate were used for drying . The EOs were stored in the dark sealed vials at 4 °C until starting the experiment.

Plant essential oils analysis

Chemical composition of plant was analyzed using gas chromatography mass spectrometer. The plants components was performed by comparing their retention times and mass spectra from Wiley library [9].

Results

The chemical constituents of *Tanacetum balsamita* and *Artemisia sieberi* which showed the highest efficacy for larviciding. Results showd a of 39 constituents from *T. balsamita*. The main constituents were Thujone (52.37%) and Carvone (26.84%) respectively. Totally 57 constituents were detected in the essential oil of *Artemisia sieberi* and the main components were: camphor (23.6%), 2-Ethyl-3-methyl maleic anhydride (15. 193%) and, Bombykal (10.32%), Ethylbutenol (10.74%) (Table 1,2). The values of LC50 and LC90 of EOs were 47.9 and 178.8 ppm for *A. sieberi*. The figure of 26.2and 52.4 ppm was observed for *T.balsamita* (table.3). Probit regression lines of two plants against larvae of *An.stephensi* is shown in Figs. 5,6.

Discussion

A total of 500 species of *Artemisia* are found worldwide. Asia has the greatest concentration of species, 35 species of the genus found in Iran. According to different investigations, It is used for the treatment of different diseases such as malaria, hepatitis, cancer, inflammation, and infections by fungi, bacteria, and viruses [10–13]. In the Middle east, the plant *of A. sieberi* is considered as famous medicinal plant for anthelmintic effects. The extractions of its flowers and leaves were used for treatment of gangrenous ulcers, infectious ulcers, and inflammations. *A. sieberi* is used as fodder for sheep for increasing of weight. It was used as carminative, to relieve inflammation and abscesses and to prevent leprosy. It is used as pharmacy in traditional medicine. It has anthelmintic, anti-spasmodic, antirheumatic, and antibacterial potency for the treatment of malaria, hepatitis, cancer, inflammation, and menstrual-related disorders [14]. The results of chemical composition analysis of *A. sieberi* essential oils showed the presence of β-thujone (0–11.6%), camphor (2.8–42.9%), 1,8-cineole (0.0-48.7%) and α-thujone (0-62.1%). (Mahboubi et al, 2015). *A.sieberi* extracts showed antimalarial effects against murine malaria [15].

The major constituents of the leaf oil of *T.balsamita* were bornyl acetate (47.7%), pinocarvone (27.1%), camphor (9.3%) and terpinolene (5.4%), while the flower oil contained bornyl acetate (55.2%), pinocarvone (34.2%), camphor (2.8%) and terpinolene (2.0%) and the stem oil contained bornyl actate (49.2%), pinocarvone (28%), camphor (9.5%) and terpinolene (6%). [16]. Different local plants were identified and then their extractions were evaluated against *An. stephensi* in Iran [17].

Conclusion

Due to high larvicical activities of plants against malaria vector, *An.stephensi*, it is recommended to make appropriate formulations for these plants and evaluated them under field conditions.

Declarations

Ethics approval and consent to participate:

There is not applicable

Consent for publication:

applicable

Availability of data and material:

applicable

Competing interests:

The author declare that there is no conflict of interest

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Tables

Table 1
Chemical components of *Tanacetum balsamita*

NO	tR (Minutes	compound	%	RI
1	1.774	Ethanol	0.478	-
2	2.111	Hexane	0.232	-
3	6.503	cis-Salvene	0.398	-
4	6.79	3,4-Octadiene, 7-methyl-	0.035	-
5	10.28	Comphene	0.080	873
6	11.58	beta-Pinene	0.079	904
7	12.406	1-Phenylethanol	0.140	922
8	12.975	Yomogi alcohol	1.599	933
9	14.002	p-Cymene	0.257	955
10	14.15	m-Cymene	0.188	958
11	14.409	1,8-Cineole	1.352	963
12	15.711	gamma-Terpinene	0.035	991
13	19.712	Thujone	52.379	1067
14	20.046	2,5-Octadiene	1.298	1074
15	20.144	Artemiseole	0.446	1076
16	20.461	trans-p-2,8-Menthadien-1-ol	0.242	1082
17	20.576	cis-3,7-Dimethyl-1,3,6-octatriene	0.260	1084
18	20.813	6-Nonynoic acid	0.349	1089
19	22.038	2,6-Dimethyl-1,5-heptadien-4-ol acetate	0.909	1113
20	22.648	3-Cyclohexen-1-ol, 5-methylene-6-(1-methylethenyl)-	0.208	1125
21	23.838	2-Nonynoic acid	1.796	1148
22	26.826	Carvone	26.844	1211
23	27.456	Carvone oxide	1.177	1229
24	27.932	Novatone	1.264	1243
25	29.061	4-Heptanone	0.140	1277
26	36.012	Germacrene D	1.015	1403

NO	tR (Minutes	compound	%	RI
27	36.611	tau-Muurolol	0.775	1420
28	37.201	beta-Bisabolene	1.193	1438
29	37.8	delta-Cadinene	0.369	1455
30	38.074	alpha-Guaiene	0.196	1463
31	40.471	Ledene oxide	0.717	1522
32	41.023	Veridiflorol	0.151	1533
33	42.645	alpha-Cadinol	0.783	1565
34	43.238	5-beta-H,7-beta,10-alpha-Selina-4(19),11-diene	0.783	1576
35	54.213	n-Hexadecanoic acid	0.167	1867
36	54.519	1,2-Longidione	0.599	1874
37	57.967	9-Octadecene	0.175	1955
38	58.953	Isophytol	0.314	1977
39	60.79	4H-Naphtho[2,3-b]pyran-4,6,9-trione, 5,8-dimethoxy-2-methyl- (CAS)	0.578	2020

Table 2

Chemical components of *Artemisia sieberi*

NO	tR (Minutes	compound	%	RI
1	1.7	Ethanol	0.022	
2	1.765	Acetone	0.093	
3	1.975	2-Butenal	0.067	
4	2.084	Hexane	0.510	
5	2.631	Methacrylic acid	0.485	
6	3.178	Ethylbutenol	0.035	
7	3.558	2,5-Octadiene	0.047	
8	4.247	Butanoic acid, 3-methyl-, methyl ester	0.043	
9	4.436	2-Methyl-3-butenoic acid	0.070	
10	4.533	2-Hexenal	0.034	
11	6.366	Ethyl 2-methylbutanoate	0.061	
12	6.469	Pentanoic acid, ethyl ester	0.136	
13	8.833	5-Methyl-3-octyne	0.177	837
14	9.053	alpha-Pinene	0.202	843
15	10.44	Comphene	4.142	877
16	11.465	Sabinene	0.100	902
17	11.566	beta-Pinene	0.240	904
18	12.397	Ethanol	0.419	921
19	12.932	Acetone	0.118	0.118
20	13.167	2-Butenal	0.242	937
21	13.551	Hexane	0.157	946
22	14.135	Methacrylic acid	0.420	958
23	14.73	Ethylbutenol	10.748	970
24	15.792	2,5-Octadiene	0.272	992

25	17.189	Butanoic acid, 3-methyl-, methyl ester	0.063	1018
26	18.852	Linalool	0.298	1051
27	20.978	Camphor	23.206	1092
28	21.726	Borneol	2.680	1107
29	22.247	4-Terpineol	0.835	1117
30	22.529	Furfural	0.103	1122
31	22.799	alpha-Terpineol	0.269	1128
32	22.987	Piperitol	0.641	1132
33	23.727	Terpinene-3-ol	0.921	1146
34	24.127	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, cis-	0.109	1154
35	24.37	Isobornyl acetate	0.090	1159
36	24.592	Davana oil	0.337	1163
37	25.289	Carvol	0.630	1177
38	25.991	Piperitone oxide	1.610	1191
39	27.159	Bornyl acetate	0.124	1221

40	31.23	alpha-Copaene	0.427	1323
41	32.549	cis-Jasmone	1.422	1345
42	33.159	trans-Caryophyllene	0.204	1355
43	34.849	Perilla alcohol	0.497	1382
44	35.929	Germacrene D	0.665	1400
45	36.587	Germacrene B	0.928	1420
46	37.825	Davana ether	5.029	1456
47	38.757	2-Ethyl-3-methyl maleic anhydride	15.193	1484
48	39.879	Bombykal	3.281	1511
49	41.082	Bombykal	10.324	1534
50	41.496	Bombykal	5.984	1542
51	42.004	Humuladienone	0.309	1552
52	43.297	Isoaromadendrene epoxide	0.674	1578
53	44.523	4-[3-Methyl-1-(2-methyl-1-propenyl)-2-butenyl]-3-cyclohexen-1-one	0.962	1603
54	44.971	cis-Davanone	1.179	1621
55	48.304	Ascaridole	1.274	1730
56	51.001	Benzyl salycilate	0.237	1793
57	54.213	n-Hexadecanoic acid	0.652	1867

Table 3

LC50 and LC90 values of *Artemisia sieberi* and *Tanacetum balsamita* against larvae of *An.stephensi*

plant	a	b ± SE	LC50 (ppm) ± 95%C.L.	LC90 (ppm) ± 95%C.L.	*χ2* (heterogeneity)	*χ2* table (df)	p-Value
Artemisia	-3.7649	2.2405	41.3	141.2	40.828 *	16.26	0.001
sieberi		± 0.222	47.9	178.9		6 (3)	
			55.1	248.9			
Tanacetum	-6.0418	4.2586	23.1	46.2	3.863*	13.27	0.001
balsamita		± 0.343	26.2	52.4		7 (2)	
			28.7	61.6			

Figures

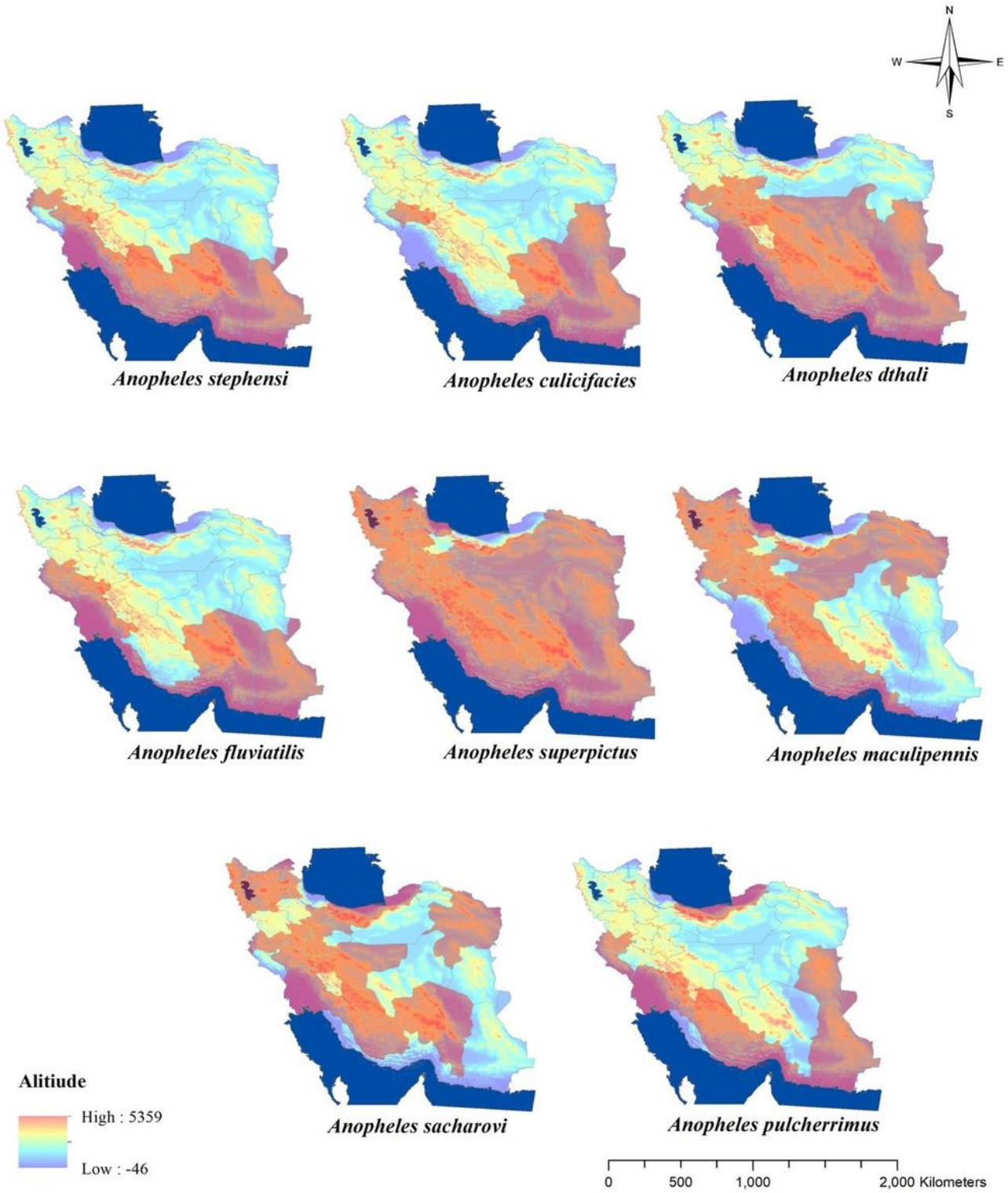

Figure 1

distribution of malaria vectors in Iran. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

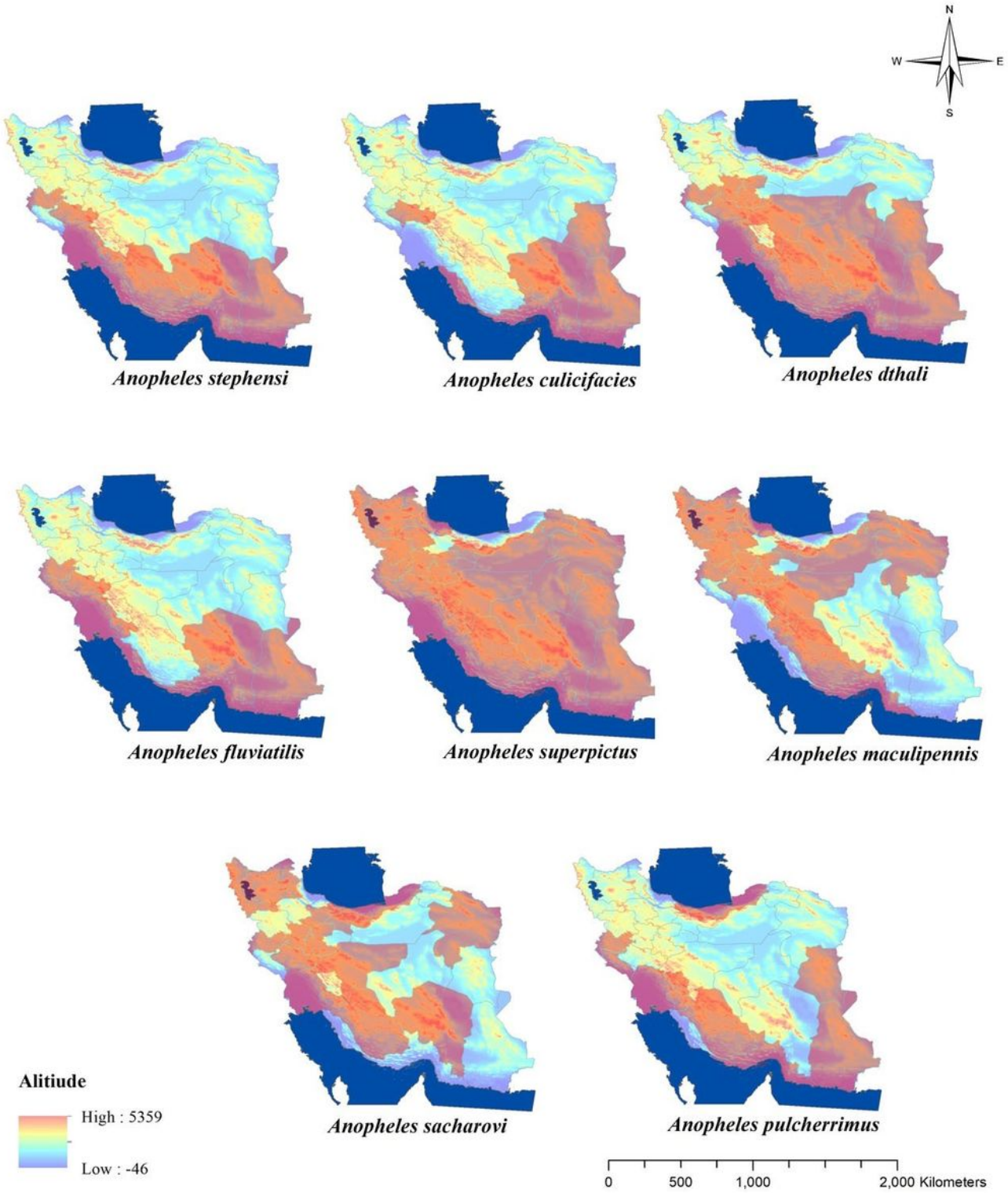

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Figure 2

Map of study area, east Azerbaijan, Iran Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

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Figure 3

Artemisia sieberi

Figure 3

Artemisia sieberi

Figure 4

Tanacetum balsamita

Figure 4

Tanacetum balsamita

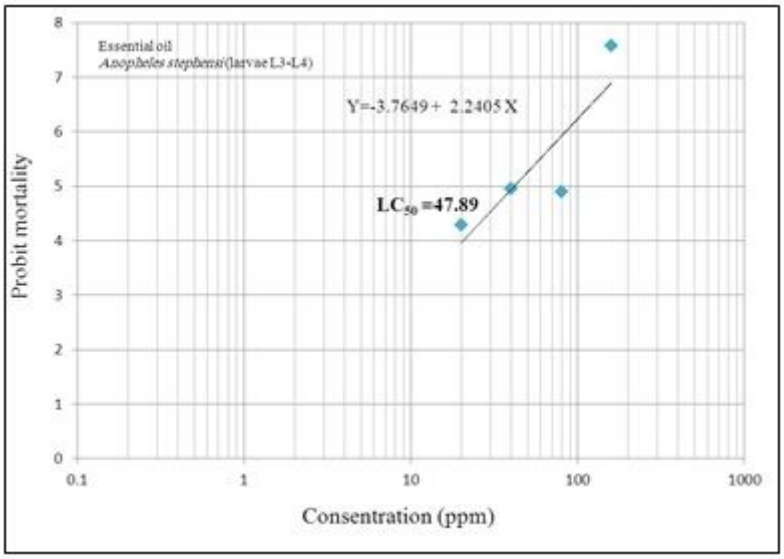
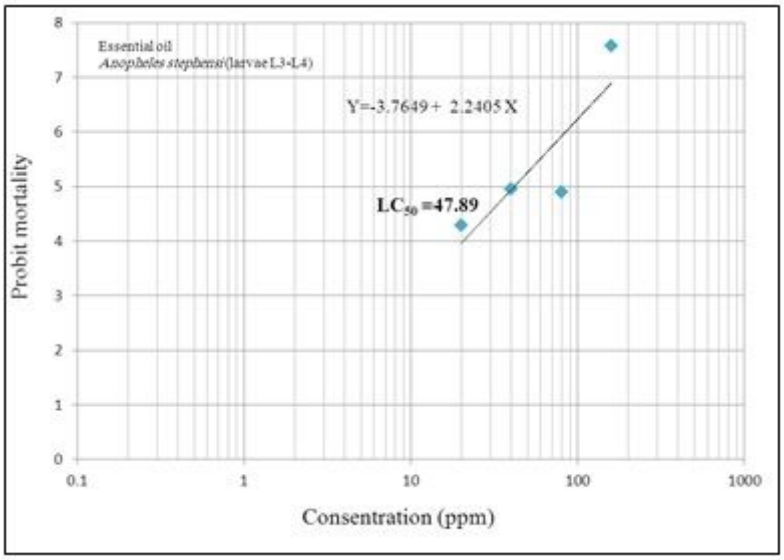


Figure 5

Probit regression line of Artemisia sieberi against larvae of An.stephensi



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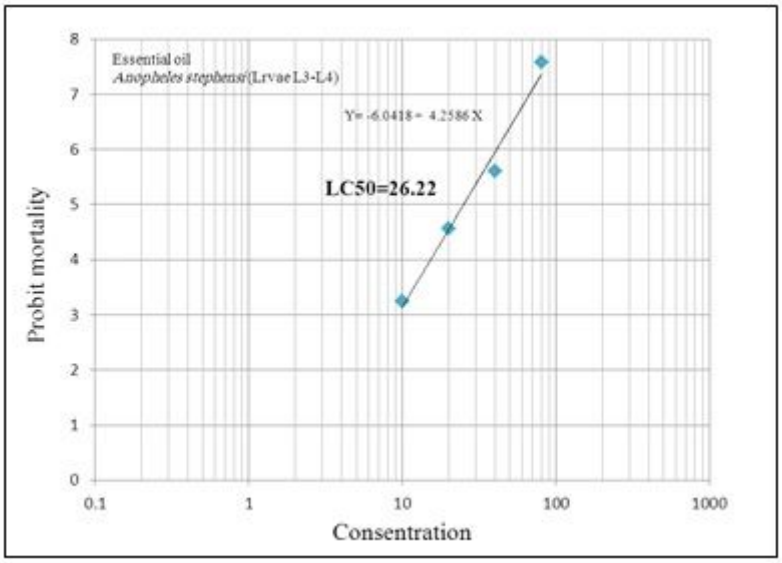

Figure 6

Probit regression line of Tanacetum balsamita against larvae of An.stephensi

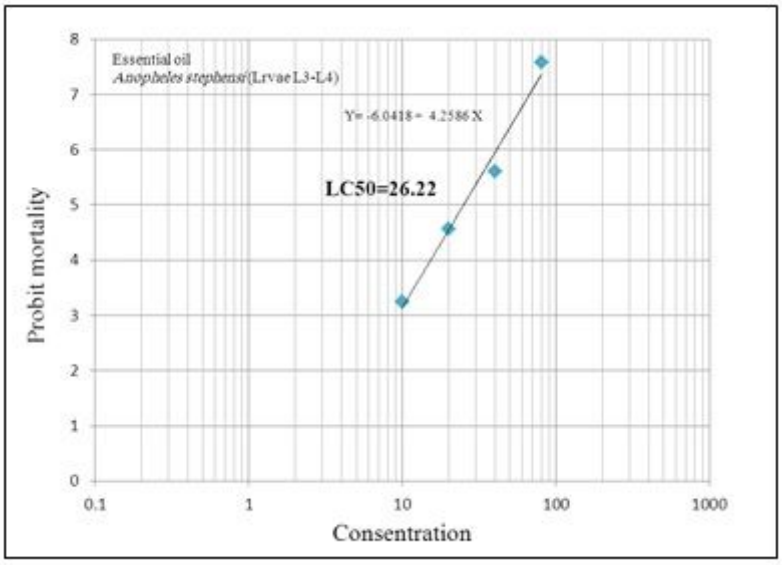

Figure 6

Probit regression line of Tanacetum balsamita against larvae of An.stephensi